The Effect of Corticosterone-Induced Stress on Amino Acid Digestibility in Ross Broilers[1,2]

W. S. Virden,* M. S. Lilburn,† J. P. Thaxton,* A. Corzo,* D. Hoehler,‡ and M. T. Kidd*[3]

*Department of Poultry Science, Mississippi State University, Mississippi State 39759-9665; †The Ohio State University, Columbus 43210; and ‡Degussa Corporation, Kennesaw, GA 30144

ABSTRACT Two experiments (Exp.) were conducted to establish amino acid (AA) digestibility coefficients (DC) for broilers given corticosterone (CS)-induced stress using the apparent ileal digestibility assay. For Exp. 1, 192 Ross × Ross 708 male broilers were placed into 16 floor pens (12 birds/pen). For Exp. 2, 120 Ross × Ross 308 male broilers were placed into 10 floor pens (12 birds/pen). Pens contained nipple drinkers, pan feeders, and softwood shavings. Both experiments were completely randomized designs using pen as the experimental unit. In both experiments, chicks were given a common starter diet from d 1 to 20. From d 21 to 30, broilers were provided a control diet or the control + 15 mg of CS/kg of diet dissolved in soybean oil (8 and 5 replications/treatment in Exp. 1 and 2, respectively). Diets were based on corn (65.07%) and soybean meal (26.36%) and contained an indigestible marker (chromic oxide 0.3%). Diets were formulated to contain 3,175 kcal of ME, 18.5% CP, 0.79% digestible TSAA, and 1.00% digestible Lys. Stress validation was accomplished by measuring BW gain, feed intake, and liver weight on d 30. Evidence that stress occurred was apparent due to the fact that broilers fed CS had lower BW gain and higher liver weight than those fed control. On d 30, the ileal contents were removed from 3 birds/pen, pooled, dried, and analyzed for AA content. Amino acid DC were calculated using the following equation: DC = 100 − (dietary marker % × ileal AA %) / (ileal marker % × dietary AA %) × 100. Amino acid digestibility did not differ ($P > 0.05$) between treatments in either experiment. Based on this research, future research should be directed at establishing DC for other nutrients in stressed broilers or the effect of different nutrients on the stress response.

Key words: stress, corticosterone, digestibility, amino acid, broiler

2007 Poultry Science 86:338–342

INTRODUCTION

Of the many challenges facing broiler production, one of the most significant is physiological stress. When broilers are exposed to stressors for extended durations, the birds must acclimate to the stressor rather than attempt to combat or avoid it. This is accomplished by the activation of the hypothalamic-pituitary-adrenal system (Siegel, 1980). Integration of the hypothalamic-pituitary-adrenal system stimulates the hypothalamus to produce corticotropin releasing factor, which causes the pituitary gland to release adrenocorticotropic hormone (**ACTH**; Holmes and Phillips, 1976). The presence of ACTH in the bloodstream stimulates the adrenals to secrete corticosterone (**CS**; Holmes and Phillips, 1976). Chronically elevated CS levels result in many deleterious effects to the bird's performance, with one of the most detrimental being the catabolism of structural protein through CS-induced gluconeogenesis (Baxter and Rousseau, 1979). This shift in metabolism is well documented in birds treated with CS or ACTH, which typically demonstrate significant decreases in BW (Dulin, 1956; Garren et al., 1961; Freeman and Manning, 1975; Bartov et al., 1980; Thaxton et al., 1982; Siegel and van Kampen, 1984; Davison et al., 1985; Klasing et al., 1987; Siegel et al., 1989; Puvadolpirod and Thaxton, 2000a,b,c,d; Post et al., 2003). Further, the decrease in BW has been shown to be independent of increased feed intake (Bartov et al., 1980; Siegel and van Kampen, 1984; Puvadolpirod and Thaxton, 2000d). Research such as these previously mentioned studies involving physiological stress induced through exogenous administration of stress hormones demonstrates a general model for stress research because the same hormonal cascade occurs during the stress response regardless of the specific stressor responsible for the initiation of the sequence. However, this type of research is probably more closely associated with environmental, non-temperature-related stressors because heat stress almost always results in a reduction in feed intake (Austic, 1985).

Received June 27, 2006.

Accepted October 28, 2006.

[1]This is Journal Article Number J-10967 from the Mississippi Agricultural and Forestry Experiment Station supported by MIS-322230.

[2]Use of trade names in this publication does not imply endorsement by the Mississippi Agricultural and Forestry Experiment Station of the products, nor similar ones not mentioned.

[3]Corresponding author: mkidd@poultry.msstate.edu

One possible means of learning how to better combat stress is to conduct research delineating the effects of stress on nutrient digestibility. This knowledge could aid in the nutritionist's ability to establish nutrient requirements for stressed broilers. Unfortunately, very little research of this type has been conducted. One such study by Puvadolpirod and Thaxton (2000d) examined the nutrient digestibility of broilers given ACTH-dispensing implants. These researchers concluded that broilers treated with ACTH had significantly lower protein and carbohydrate digestibility than nonstressed broilers (Puvadolpirod and Thaxton, 2000d).

More recently, much attention has been given to estimating amino acid (**AA**) digestibility using the apparent ileal digestibility assay (Ravindran et al., 1999). The ileal digestibility assay entails the removal of the ileum from the bird, followed by the removal of its contents (Ravindran et al., 1999). The ileal contents can then be dried, digested, and analyzed for nutrient content using HPLC (Ravindran et al., 1999). This assay has several advantages. For example, it is easy to obtain ileal contents (i.e., sacrifice bird and remove ileal contents), it can be performed on chicks, and it is very precise with low variability (Lemme et al., 2004). Furthermore, it is not necessary to monitor feed intake or force feed birds because this assay is able to utilize the inclusion of an indigestible marker (i.e., chromic oxide, titanium dioxide, or ash insoluble ash) in the treatment diet (Kadim et al., 2002). One of the most important advantages of this assay is that because the digesta is obtained from the ileum, variation is reduced because contamination from hindgut fermentation and urinary waste does not occur (Lemme et al., 2004).

If more research were conducted to contribute to the understanding of the effects of stress on AA digestibility, perhaps nutritionists could better estimate AA requirements for periods when known stressors are present. Thus, the purpose of this research was to examine the effects of CS-induced stress on AA digestibility in Ross broilers using the apparent ileal digestibility assay.

MATERIALS AND METHODS

Husbandry and Treatments

This research consisted of 2 experiments (**Exp.**). Experiments 1 and 2 differed very little. In Exp. 1, 192 Ross × Ross 708 male broilers were placed into 16 pens of a floor pen facility (12 birds/pen). In Exp. 2, 120 Ross × Ross 308 male broilers were placed into 10 pens of a floor pen facility (12 birds/pen). Other than the former noted differences, both experiments were identical. Each pen contained 1 Chore-Time tube feeder (Chore-Time Poultry Production Systems, Milford, IN), a nipple drinker line (4 nipples/pen), and built-up soft-wood shavings. From d 1 to 20, chicks were fed a common starter diet (Table 1), which exceeded the AA requirements established by the NRC (1994). On d 21, broilers and feed were weighed by pen, and treatment feed was administered from d 21 to 30. Treatments consisted of 1) control diet; 2) control diet plus 15 mg CS/kg of diet. Addition of CS to the feed was accomplished by dissolving CS into soybean oil. Previous experimentation to validate this stress model led to the usage of the CS inclusion level herein (Virden et al., 2005). Experiments 1 and 2 had 8 replications/treatment and 5 replications/treatment, respectively. At 30 d of age, broilers and feed were weighed by pen to calculate BW gain, feed intake, and feed conversion. Furthermore, 3 birds per pen were selected at random and killed by cervical dislocation. The liver was removed from each bird, pooled by pen, and weighed to obtain a wet liver weight. The livers were dried in a drying oven for approximately 7 d and then weighed to obtain a dry liver weight. Also, ileal contents from these same 3 birds were obtained from each bird, pooled by pen, then analyzed for AA digestibility.

Table 1. Composition of test diets for experiments (Exp.) 1 and 2

Ingredient	Common starter diet[1]	Test grower diet[2]
	(%)	
Corn	51.63	65.07
Soybean meal	39.06	26.36
Poultry fat	5.41	3.27
Dicalcium phosphate	1.85	1.68
Limestone	1.09	1.02
Filler oil[3]	0.00	1.00
NaCl	0.51	0.51
Chromic oxide[4]	0.00	0.30
Premix[5]	0.25	0.25
DL-Methionine	0.16	0.25
L-Lysine HCl	0.00	0.17
Choline Cl	0.03	0.06
L-Threonine	0.00	0.05
Calculated analysis[6]		
CP, %	23.00	18.50
ME, kcal/kg	3,100.00	3,175.00
Digestible Lys, %	1.21	1.00
Digestible TSAA, %	0.89	0.79
Digestible Thr, %	0.79	0.67
Calcium, %	0.94	0.84
Available P, %	0.47	0.42
Na, %	0.22	0.22

[1]Common starter diet was fed to broilers from 1 to 20 d of age during Exp. 1 and 2.

[2]The test grower diet was fed to broilers from 21 to 30 d during Exp. 1 and 2 (i.e., during stress administration). Corticosterone was added (15 mg/kg of diet) at the expense of filler oil from 21 to 30 d.

[3]Filler oil represented food grade soybean oil.

[4]Chromic oxide is an indigestible marker used to insure that the digesta samples obtained for analysis came from the feed of interest.

[5]Broiler premix contained the following per kilogram of diet: vitamin A (vitamin A acetate) 7,718 IU; cholecalciferol 2,200 IU; vitamin E (source unspecified) 10 IU; menadione, 0.9 mg; B_{12} 11 μg; choline, 379 mg; riboflavin, 5.0 mg; niacin, 33 mg; D-biotin, 0.06 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; Mn, 55 mg; Zn, 50 mg; Fe, 28 mg; Cu, 7 mg; I, 1 mg; Se, 0.2 mg.

[6]Analyzed values for individual amino acids in the grower diet were: Lys, 1.25%; Met, 0.55%; Cys, 0.36%; TSAA, 0.95%; Thr, 0.82%; Arg, 1.36%; Ile, 0.86%; Leu, 1.76%; Val, 0.94%; His, 0.53%; Phe, 1.02%; Gly, 0.85%; Ser, 1.03%; Pro, 1.19%; Ala, 1.03%; Asp, 2.11%; and Glu, 3.84%.

Apparent Ileal Digestibility Assay

This research utilized the apparent ileal digestibility assay for AA digestibility determinations (Lemme et al.,

Table 2. Live performance of broilers fed corticosterone (CS) in experiment 1[1]

Parameter	CS 0 mg/kg	CS 15 mg/kg	SEM	Probability
BW gain (kg) 21 to 30 d	0.689[a]	0.560[b]	0.0124	<0.0001
Feed intake (g/bird/d) 21 to 30 d	116.9[b]	127.9[a]	2.03	0.002
Feed conversion (kg/kg) 21 to 30 d	1.70[b]	2.29[a]	0.033	<0.0001
Wet liver weight (g) 30 d of age	116.4[b]	147.0[a]	3.72	<0.0001
Dry liver weight (g) 30 d of age	28.2[b]	49.2[a]	2.05	<0.0001

[a,b]Means within the same row with no common superscript differ ($P \leq 0.05$).

[1]Corticosterone treatments were administered from 21 to 30 d.

2004). Briefly, chromic oxide was added to the test diets in both experiments at a level of 0.3% as an indigestible marker. Broilers were given ad libitum access to food and water at all times. As previously mentioned 3 birds per pen were killed in both experiments, and ileums (i.e., the section of the small intestine beginning at a point directly below the Meckel's diverticulum and ending at a point approximately 4 cm anterior to the ileo-cecal junction) were removed. The digesta were removed from each ileum and pooled by pen. The digesta samples were dried for approximately 3 d in a forced air drying oven at 65°C. The ileal contents were then ground into a fine powder using a mortar and pestal. After the necessary chemical analyses were performed to determine the AA and Cr levels in the test diet and the digesta samples, apparent digestibility coefficients (**DC**) were calculated using the following equation: DC = 100 − (Cr diet × AA contents/Cr digesta × AA diet) × 100.

Chemical Analysis

Amino acid levels in the dietary treatments and ileal samples were determined by following the procedures described by Llames and Fontaine (1994). Briefly, composite samples of the test diets and digesta were analyzed by HPLC after acid hydrolysis. The TSAA degradation of the test diet and digesta samples was circumvented by treating samples with performic acid prior to hydrolysis. This step facilitates the conversion of Met and Cys to Met sulfone and cysteic acid (Llames and Fontaine, 1994). These compounds can be analyzed using HPLC, allowing for the calculation of the Met and Cys contents of the samples in accordance with AOAC 994.12 (Llames and Fontaine, 1994).

Table 3. Live performance of broilers fed corticosterone (CS) in experiment 2[1]

Parameter	CS 0 mg/kg	CS 15 mg/kg	SEM	Probability
BW gain (kg) 21 to 30 d	0.645[a]	0.373[b]	0.0209	<0.0001
Feed intake (g/bird/d) 21 to 30 d	163.7	175.7	3.86	0.059
Feed conversion (kg/kg) 21 to 30 d	2.03[b]	3.81[a]	0.151	<0.0001
Wet liver weight (g) 30 d of age	135.9[b]	200.0[a]	11.73	0.005
Dry liver weight (g) 30 d of age	33.0[b]	69.8[a]	5.07	0.0009

[a,b]Means within the same row with no common superscript differ ($P \leq 0.05$).

[1]Corticosterone treatments were administered from 21 to 30 d.

Table 4. Amino acid digestibility coefficients of broilers fed corticosterone (CS)[1]

Amino acid[2]	Digestibility coefficient[2] CS (0 mg/kg) (%)	CS (15 mg/kg) (%)	SEM	Probability
Met	89.88	89.71	0.70	0.83
Cys	66.93	67.21	1.55	0.90
TSAA	81.02	81.06	1.07	0.98
Lys	85.12	85.26	0.93	0.92
Thr	68.78	67.51	1.67	0.60
Arg	81.27	80.51	0.99	0.59
Ile	77.18	77.31	1.21	0.94
Leu	81.23	81.28	1.05	0.98
Val	74.75	74.64	1.26	0.95
His	80.24	80.10	1.26	0.93
Phe	81.47	80.61	1.40	0.67
Gly	70.54	70.47	1.41	0.97
Ser	76.39	76.67	1.28	0.88
Pro	76.02	75.86	1.17	0.92
Ala	75.15	74.97	1.52	0.93
Asp	77.56	77.89	1.10	0.84
Glu	85.28	85.67	0.79	0.72

[1]Corticosterone treatments were administered from 21 to 30 d.

[2]Indicates value calculated using the following equation: digestibility coefficient (%) = 100 − {(indigestible $\text{marker}_{\text{diet}}$ × amino $\text{acid}_{\text{excreta}}$)/(indigestible $\text{marker}_{\text{excreta}}$ × amino $\text{acid}_{\text{diet}}$) × 100}. Digesta and diet samples were analyzed to obtain values used herein.

[3]Represents pooled data from Exp. 1 and 2.

Statistical Analysis

Both experiments were completely randomized designs using pen as the experimental unit. All data were analyzed using the GLM procedure of SAS Institute (1998). Means differing significantly were separated using the LSMEANS procedure of SAS Institute (1998).

RESULTS

In both experiments, broilers fed diets containing CS had significantly lower ($P < 0.0001$) BW gain than broilers fed the control diet (Tables 2 and 3). Feed intake ($P < 0.01$ in Exp. 1; $P = 0.06$ in Exp. 2) and feed conversion ($P < 0.01$ in both experiments) were higher in broilers fed diets containing CS than in those fed the control diet. Broilers fed diets containing CS had higher ($P < 0.0001$) wet and dry liver weights than birds fed the control diet. The AA DC did not differ between treatments in either experiment (Table 4).

DISCUSSION

The effects of CS on BW gain in both experiments are not surprising as similar results are well documented (Dulin, 1956; Garren et al., 1961; Freeman and Manning, 1975; Bartov et al., 1980; Thaxton et al., 1982; Siegel and van Kampen, 1984; Davison et al., 1985; Klasing et al., 1987; Siegel et al., 1989; Puvadolpirod and Thaxton, 2000a,b,c,d; Post et al., 2003). Furthermore, the increases in feed intake and feed conversion due to stress administration in this research are in agreement with findings of previous researchers (Bartov et al., 1980; Siegel and van Kampen, 1984; Puvadolpirod and Thaxton, 2000d). Wet and dry liver weights were increased due to CS administration in both experiments. This is in agreement with previous research utilizing CS or ACTH to induce physiological stress (Gross et al., 1980; Siegel and van Kampen, 1984; Donker and Beuving, 1989; Covasa and Forbes, 1995; Puvadolpirod and Thaxton, 2000a,b,c; Malheiros et al., 2003). Hence, liver lipids have been shown to increase significantly in broilers treated with ACTH (Puvadolpirod and Thaxton, 2000a,b). In this research, differences in AA DC due to physiological stress were not present in either experiment. This is in contrast to research presented by Puvadolpirod and Thaxton (2000d). These researchers found a significant depression in protein digestibility in broilers given ACTH-dispensing implants (Puvadolpirod and Thaxton, 2000d). One reason for this discrepancy could be the differences in digestibility analysis methods. Puvadolpirod and Thaxton (2000d) calculated nutrient digestibility by dividing nutrient intake per day by nutrients excreted per day, multiplying the quotient by 100, and then dividing the quotient by nutrient intake per day. Excreta protein contents were obtained by determining total nitrogen using the Kjeldahl procedure, then calculating for protein content (Puvadolpirod and Thaxton, 2000d). Uric acid excretion has been shown to increase in ACTH and CS-treated poultry as a result of CS-induced muscle catabolism (Brown et al., 1958; Adams, 1968; Siegel and van Kampen, 1984). The current research utilized ileal contents as opposed to excreta for digestibility determinations. As such, urinary AA contributions were not measured. Time of measurement may have played a role in the results obtained; the current research measured digestibility on d 30. Puvadolpirod and Thaxton (2000d) collected feces in 2-d intervals and measured digestibility accordingly. Subsequent research conducted by the same laboratory on laying hens injected with ACTH, which utilized the same digestibility assay, demonstrated no effects on protein digestibility (Odihambo Mumma et al., 2006). The AA DC values obtained in this research are comparable with those for corn and soybean meal determined by previous researchers (Ravindran et al., 1999; Kadim et al., 2002; Lemme et al., 2004). However, an exact comparison cannot be made because contributions from other ingredients in the corn and soybean meal diet used to obtain the values presented herein could have caused the slight variations in digestibility estimates when compared with previous research. It is important to point out that the DC presented in this research were not corrected for endogenous AA losses.

Based on these findings, the AA digestibility of Ross × Ross 308 and 708 broilers should not be affected by CS-induced stress at the dosage level utilized herein. Thus environmental stressors, other than temperature, should not affect AA digestibility. Future research should focus on nutrient (e.g., AA) utilization during and after periods of physiological stress or AA digestibility in the presence of specific stressors.

REFERENCES

Adams, B. M. 1968. Effect of cortisol on growth and uric acid excretion in the chick. J. Endocrinol. 40:145–151.

Austic, R. E. 1985. Feeding poultry in hot and cold climates. Pages 123–136 in Stress Physiology in Livestock. M. K. Yousef, ed. CRC Press, Boca Raton, FL.

Bartov, I., L. S. Jensen, and J. R. Veltman. 1980. Effect of corticosterone and prolactin on fattening in broiler chicks. Poult. Sci. 59:1328–1334.

Baxter, J. D., and G. G. Rousseau. 1979. Glucocorticoid hormone action: An overview. Pages 1–24 in Glucocorticoid Hormone Action. J. D. Baxter and G. G. Rousseau, ed. Springer-Verlag, Heidelberg, Germany.

Brown, K. I., D. S. Brown, and R. K. Meyer. 1958. The effect of surgical trauma, ACTH, and adrenal cortical hormones on electrolytes and gluconeogenesis in male chickens. Am. J. Physiol. 192:43–50.

Covasa, M., and J. M. Forbes. 1995. Selection of foods by broiler chickens following corticosterone administration. Br. Poult. Sci. 36:489–501.

Davison, T. E., B. M. Freeman, and J. Rea. 1985. Effects of continuous treatment with synthetic ACTH1 – 24 or corticosterone on immature *Gallus domesticus*. Gen. Comp. Endocrinol. 59:416–423.

Donker, R. A., and G. Beuving. 1989. Effect of corticosterone infusion on plasma corticosterone concentration, antibody production, circulating leukocytes and growth in chicken lines selected for humoral immune responsiveness. Br. Poult. Sci. 30:361–369.

Dulin, W. E. 1956. Effects of corticosterone, cortisone and hydrocortisone on fat metabolism in the chick. Proc. Soc. Exp. Biol. Med. 92:253–255.

Freeman, B. M., and A. C. C. Manning. 1975. The response of the immature fowl to multiple injections of adrenocorticotropic hormone. Br. Poult. Sci. 16:212–219.

Garren, H. W., C. H. Hill, and M. W. Carter. 1961. Adrenal response of young chickens to adrenocorticotropic hormone as influenced by dosage and frequency of injection. Poult. Sci. 40:446–453.

Gross, W. B., P. B. Siegel, and R. T. DuBose. 1980. Some effects of feeding corticosterone to chickens. Poult. Sci. 59:516–522.

Holmes, W. N., and J. G. Phillips. 1976. The adrenal cortex of birds. Pages 292–420 in General, Comparative and Clinical Endocrinology of the Adrenal Cortex. I. Chester-Jones and I. W. Henderson, ed. Academic Press, New York, NY.

Kadim, I. T., P. J. Moughan, and V. Ravindran. 2002. Ileal amino acid digestibility assay for the growing meat chicken—Comparison of ileal and excreta amino acid digestibility in the chicken. Br. Poult. Sci. 44:588–597.

Klasing, K. C., D. E. Laurin, and D. M. Fry. 1987. Immunologically mediated growth depression in chicks: Influence of feed intake, corticosterone and interleukin-1. J. Nutr. 117:1629–1637.

Lemme, A., V. Ravindran, and W. L. Bryden. 2004. Standardized ileal amino acid digestibility of raw materials in broilers. Proc. Multi-State Poult. Meeting, 2004. Indianapolis, IN.

Llames, C. R., and J. Fontaine. 1994. Determination of amino acids in feeds: Collaborative study. J. AOAC Int. 77:1362–1402.

Malheiros, R. D., V. M. B. Moraes, A. Collin, E. Decuypere, and J. Buyse. 2003. Free diet selection by broilers as influenced by dietary macronutrient ratio and corticosterone supplementation. 1. Diet selection, organ weights, and plasma metabolites. Poult. Sci. 82:123–131.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. 9th Rev. ed. Natl. Acad. Press, Washington, DC.

Odihambo Mumma, J., J. P. Thaxton, Y. Vizzier-Thaxton, and W. L. Dodson. 2006. Physiological stress in laying hens. Poult. Sci. 85:761–769.

Post, J., J. M. Rebel, and A. A. H. M. ter Huurne. 2003. Physiological effects of elevated plasma corticosterone concentrations in broiler chickens. An alternative means by which to assess the physiological effects of stress. Poult. Sci. 82:1313–1318.

Puvadolpirod, S., and J. P. Thaxton. 2000a. Model of physiological stress in chickens: 1. Response parameters. Poult. Sci. 79:363–369.

Puvadolpirod, S., and J. P. Thaxton. 2000b. Model of physiological stress in chickens: 2. Dosimetry of adrenocorticotropin. Poult. Sci. 79:370–376.

Puvadolpirod, S., and J. P. Thaxton. 2000c. Model of physiological stress in chickens: 3. Temporal patterns of response. Poult. Sci. 79:377–382.

Puvadolpirod, S., and J. P. Thaxton. 2000d. Model of physiological stress in chickens: 4. Digestion and Metabolism. Poult. Sci. 79:383–390.

Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. Br. Poult. Sci. 40:266–274.

SAS Institute. 1998. SAS User's Guide: Statistics Version 7.0. SAS Institute, Cary, NC.

Siegel, H. S. 1980. Physiological stress in birds. Bioscience 30:529–533.

Siegel, H. S., W. B. Gross, and E. A. Dunnington. 1989. Effects of dietary corticosterone in young Leghorn and meat-type cockerels. Br. Poult. Sci. 30:185–192.

Siegel, H. S., and M. van Kampen. 1984. Energy relationships in growing chickens given daily injections of corticosterone. Br. Poult. Sci. 25:477–485.

Thaxton, J. P., J. Gilbert, P. Y. Hester, and J. Brake. 1982. Mercury toxicity as compared to adrenocorticotropin-induced physiological stress in the chicken. Arch. Environ. Contam. Toxicol. 11:509–514.

Virden, W. S., C. D. Zumwalt, J. P. Thaxton, A. Corzo, W. A. Dozier III, and M. T. Kidd. 2005. Comparison of two models for induction of controlled stress in broilers. Poult. Sci. 84(Suppl. 1):140.